

Alzheimer's disease-associated CSF biomarkers do not correlate with CSF volumes or CSF production rate

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Abstract

Background: Neuropathologically, Alzheimer's disease (AD) is characterised by accumulation of a 42 amino acid peptide called amyloid β ($A\beta_{42}$) in extracellular senile plaques together with intraneuronal inclusions of hyperphosphorylated tau protein in neurofibrillary tangles and neuronal degeneration. These changes are reflected in the cerebrospinal fluid (CSF), the volumes and production rates of which vary considerably between individuals, by reduced concentration of $A\beta_{42}$, increased concentration of phosphorylated tau (P-tau) protein and increased concentration of total tau (T-tau) protein, respectively.

Findings: We addressed the outstanding question if CSF concentrations of $A\beta_{42}$, P-tau and T-tau, as well as a number of other AD-related CSF biomarkers, are influenced by variations in subcortical, ventricular and spinal CSF volumes, as assessed by magnetic resonance imaging (MRI) volumetry, in 19 cognitively normal healthy volunteers (mean age 70.6, SD 3.6 years). We also assessed the potential association of these biomarkers with production rates of CSF. Negative correlations were seen between the concentrations of three CSF biomarkers (albumin ratio, $A\beta_{38}$, and $A\beta_{40}$), and ventricular CSF volume, but apart from this finding, no significant correlations were observed.

Conclusions: These results speak against inter-individual variations in CSF volume and production rate as important confounds in the AD biomarker research field.

Key words: Cerebrospinal fluid; Volume; Production rate; Biomarkers; Alzheimer's disease

Background

Cerebrospinal fluid (CSF) biomarkers are increasingly used in research on and the clinical evaluation of patients with Alzheimer's disease (AD), a common neurodegenerative disorder characterized by accumulation of senile plaques and neurofibrillary tangles in the brain and progressive neuroaxonal degeneration [1]. Senile plaques are mainly composed of a 42 amino acid aggregation-prone peptide called amyloid β ($A\beta_{42}$). This peptide can be measured in the CSF; low levels reflect $A\beta$ pathology in the brain, which sequesters newly produced $A\beta_{42}$ with lower levels being able to diffuse into the CSF. Neurofibrillary tangles are intraneuronal aggregates composed of hyperphosphorylated forms of the intra-axonal protein tau (P-tau). Neurons with such inclusions eventually die and release P-tau into the CSF. AD patients thus typically have increased CSF P-tau levels, which is the most AD-specific CSF biomarker finding. Neuroaxonal degeneration and loss in AD is reflected in a general manner in the CSF by increased levels of total tau (T-tau), *i.e.*, tau determined by assays that measure all forms of tau, irrespective of phosphorylation state. A typical pattern of CSF biomarker changes in AD is thus decreased levels of $A\beta_{42}$ and increased levels of P-tau and T-tau [2].

$A\beta_{42}$ is produced from $A\beta$ precursor protein (APP), a transmembraneous protein with one transmembrane domain, a large extracellular domain and a smaller intracellular domain, by sequential cleavages of two enzymes, called β - and γ -secretase [3]. β -Secretase-mediated APP cleavage results in the release of a soluble fragment of APP called sAPP β . The remaining stub of APP then undergoes γ -secretase cleavage which splits the molecule into (i) $A\beta$ fragments of varying length, the most well-studied being $A\beta_{38}$, $A\beta_{40}$ and $A\beta_{42}$, that are secreted, and (ii) the APP intracellular domain (AICD), which may function as an intracellular secondary messenger and transcription factor. $A\beta_{42}$ is self-adhesive and may initiate self-perpetuating $A\beta$ aggregation in a prion-like manner [4]. $A\beta_{38}$ and $A\beta_{40}$ are less aggregation-prone and easier to clear from the brain parenchyma into the CSF. If APP is not cleaved by β -secretase, it may enter another processing pathway in which α -secretase cleaves the protein in the middle of the $A\beta$ domain, which results in the release of a soluble fragment called sAPP α . All these molecules can be measured in CSF and are, with the exception of $A\beta_{42}$, typically unchanged in AD [2].

CSF T-tau, P-tau and $A\beta_{42}$ may now be used to support a diagnosis of mild cognitive impairment or dementia due to AD according to revised diagnostic research criteria [5-8]. This has made it essential to learn more about potential confounding factors when these

biomarkers are assessed. Several pre-analytical and analytical factors, *e.g.*, type of CSF collection tube, aliquot volume and assay format, have been identified, which has resulted in the establishment of standard operating procedures for the whole procedure, from sampling to analysis [9]. However, two potential confounders have to our knowledge not been examined in detail before: CSF volumes and production rate, for which there are known and quite substantial inter-individual variations [10-12]

Methods

Subjects

Volunteers born 1930 to 1942 were randomly recruited through the population registry of the City of Gothenburg and the Swedish retired people's organisation between 2005 and 2007. Volunteers with neurological disorders, psychiatric illness, nephropathy, back problems, spinal operation, drug or alcohol abuse, contrast agent hypersensitivity or claustrophobia were excluded from the study during the recruitment process or the following investigation by two experienced neurologists. No individual was on treatment with any psychopharmacological drugs or centrally working analgesics. Basic blood tests measuring full blood count, sodium, potassium, blood sugar, calcium, sedimentation rate, kidney- and liver function were performed and found normal. Twenty-two healthy individuals were originally included in the study but 3 were excluded due to blood contamination of the CSF sample at the lumbar puncture, resulting in a final study group of 19 subjects (9 men and 10 women) with a mean age (SD) of 70.4 (3.7) years (Table 1).

Ethics

The Regional Ethics and Radiation Protection Committee in Gothenburg approved the study and informed consent was obtained from all participants.

ICP, CSF production rate and CSF sampling

CSF was collected by lumbar puncture (LP) in the L3/L4 interspace. All lumbar punctures were performed at approximately 11 am with the individuals placed in the left lateral recumbent position. A small pillow was placed under the head and the individuals were told to remain as still as possible. No torsion of the neck was allowed nor was speech if not urgent. The LP needle was 0.70 x 75 mm (22 gauge x 3 in.). The resting ICP was recorded for 10 minutes before CSF sampling followed by calculation of the CSF production rate as described [10, 13, 14]

Ten to twelve milliliters of CSF were collected in polypropylene tubes to minimize adsorbance of proteins to the test tube wall, aliquoted and stored at -80°C pending analysis. Prior to freezing, a small CSF aliquot was subjected to cell counting. More than 500 erythrocytes per μL of CSF were considered a significant blood contamination that might influence the biomarker results and led to exclusion from the study (3 out of 22 originally included subjects were excluded in this manner). Serum was collected by venepuncture at the same time as the spinal tap.

Imaging

Magnetic resonance imaging (MRI) was performed with a Philips Gyroscan Intera 1.5T MR system (R11.1, R1.5.4 and R2.1) using the manufacturer's synergi-spine 5 element surface coil (Philips, Eindhoven, The Netherlands). Manual segmentation of the intracranial, ventricles and brain volumes were performed on the magnetic resonance diffusion-weighted images data using QBrain software (Version 2.0; Medis Medical Imaging Systems BV, Leiden, the Netherlands). Whole-brain diffusion-weighted images were collected using a spin echo pulse sequence with the following parameters: 25 slices, slice thickness 4 mm, interslice gap 1.5 mm, repetition time 3760 ms, echo time 90 ms, 3 averages, field of view 230×230 mm, acquisition matrix 160×112 , reconstructed matrix 256×256 .

Ventricular volumes included the volume of the lateral, third and fourth ventricles.

Intracranial and brain volumes were the volume of the intracranial cavity and brain tissue (white and grey matter) from the foramen of magnum to the vertex. The intracranial subarachnoid CSF volume was computed by subtracting brain and ventricular volumes from the intracranial volume. Local thresholding was used to segment and define the edges of each intracranial volume as described in previous studies [15]. QBrain computed automatically the volumes as the sum of the segmented areas multiplied by the sum of slice thickness and intersection gap. Spinal volumes were retrieved from the images as previously described and have been reported before [16]. The operator was blinded from the CSF biomarkers findings.

Biochemical measurements

Albumin levels in serum and CSF were measured by immunonephelometry on a Beckman Immage Immunochemistry system (Beckman Instruments, Beckman Coulter, Brea, CA, USA). The albumin ratio was calculated as CSF albumin (mg/L)/serum albumin (g/L) and was used as a measure of the blood-brain barrier function. T-tau was measured using a sandwich ELISA (INNOTEST hTAU-Ag, Fujirebio, Ghent, Belgium) specifically constructed

to measure all tau isoforms irrespective of phosphorylation status. Tau phosphorylated at threonine 181 (P-tau) was measured using a sandwich ELISA (INNOTEST Phospho-Tau[181P], Fujirebio). A β -related biomarkers (A β 38, A β 40, A β 42, secreted amyloid precursor protein α [sAPP α] and secreted amyloid precursor protein β [sAPP β]) were analyzed using Meso Scale assays (Meso Scale Discovery, Rockville, MD, USA) according to kit inserts. All samples were analyzed on the same plates using the same batch of reagents by board-certified laboratory technicians who were blinded to clinical information. Intra-assay coefficients of variation were below 10% for all analytes.

Statistics

Correlations were measured using Spearman's rho (r_s) while Mann-Whitney U test was employed for group comparisons. Prism 6 (GraphPad Software, La Jolla, CA, USA) was used for all statistical analysis and a p-value of less than 0.05 was considered significant.

Results

Demographics and gender differences

There were no differences in ICP, CSF volumes, CSF production rate or CSF biomarker levels between men and women, except for intracranial subarachnoid volumes which were lower in the female group (mean 244, SD 58 mL) compared to the males (mean 298, SD 36 mL; $p=0.026$). Only the intracranial subarachnoid volumes were associated with age ($r_s=0.59$, $p=0.01$).

Tau markers

CSF concentrations of T-tau and P-tau did not correlate with ICP, CSF volumes or CSF production rate (Figure 1A and B).

Blood-brain barrier function

CSF albumin concentration volume did not correlate with ICP, CSF volumes or CSF production rate, while CSF/serum albumin ratio, the best established biomarker for blood-brain barrier function, correlated with the spinal CSF volume, (Figure 1C and D and Figure 2A).

A β -related markers

CSF concentrations of A β 38, A β 40, A β 42, sAPP α or sAPP β did not correlate with ICP, CSF production rate or spinal or intracranial subarachnoid CSF volumes. However, negative correlations were seen between the ventricular CSF volume and both A β 38, and A β 40 (Figure 1E-I and Figure 2B-C).

Discussion

This is to our knowledge the first study addressing the potential association of AD-related CSF biomarkers and CSF-related biophysical variables in healthy volunteers. We detected a negative correlation of CSF A β 38, and A β 40 with ventricular CSF volume, but not with subarachnoid or spinal CSF volumes which constitute the largest part of the total CSF volume. Since the ventricular, subarachnoid and spinal CSF volumes communicate freely with each other, the negative correlations cannot be explained by mere dilution of A β . Furthermore, such a dilution effect should have been seen for tau markers as well. Instead, the results suggest that ventricular volume may correlate with brain changes that influence A β metabolism in a manner that leads to lower concentrations of the proteins in lumbar CSF. For example, periventricular hypo-metabolism in normal pressure hydrocephalus has been shown to correlate with lower levels of CSF A β 38, A β 40 and A β 42; changes that are reversed by successful shunt therapy [17]. One earlier study using Alzheimer's Disease Neuro-imaging Initiative (ADNI) data also obtained a negative correlation of ventricular CSF volume with CSF A β 42 concentration, leading the authors to hypothesize that the correlation might be explained by more A β pathology in patients with larger ventricles and that altered CSF-blood-brain barrier functions may underlie the association [18]. However, this study did not take into account the other CSF volumes or the concentrations of the non-amyloidogenic A β 38 and A β 40 peptides. Cerebral β -amyloidosis results in a selective reduction in CSF A β 42 concentration but does not change A β 38 or A β 40 concentrations (Rosén et al., *Neuromol Med* (2012) 14:65–73). When all three markers are altered in the same direction, a more general effect on A β metabolism has to be suspected. Furthermore, we detected a negative correlation of the best-established biomarker for blood-CSF barrier dysfunction, the albumin ratio [19], with the spinal CSF volume.

CSF T-tau or P-tau concentrations were not influenced by any of the CSF volumes measured, which speaks against the approach to relate CSF T-tau and P-tau concentrations to ventricular volume as a means to increase the clinical usefulness of these markers [20].

There were no correlations between CSF production rate or ICP and any of the examined CSF biomarkers speaking against these variables as potential confounds in AD biomarker studies.

We conclude that the evidence in the present study rules out a number of potential confounds in CSF biomarker studies of AD-related processed. CSF volumes and production rate show no significant correlation with most of the examined markers and the negative correlation of ventricular CSF volume with CSF A β 38, and A β 40 in addition to the negative correlation between the albumin ratio and the spinal CSF volume most likely represent something else than mere dilution and should thus probably not be corrected for.

Abbreviations

CSF: cerebrospinal fluid; T-tau: total tau; P-tau: phosphorylated tau; A β : amyloid β ; APP: A β precursor protein; ICP: intracranial pressure; AD: Alzheimer's disease; MRI: magnetic resonance imaging.

Disclosures

The work was supported by grants from the Swedish Research Council, Swedish State Support for Clinical Research (LUA-ALF), the Knut and Alice Wallenberg Foundation and the Torsten Söderberg Foundation.

Competing interests

None reported.

Authors' contributions

ME, KB, CW, MT, HZ: conceived and designed the study; ME, UA, KB, MT, HZ: performed the experiments; ME, UA, KA, AE, KB, MT, CW, HZ: analyzed the data and wrote the paper. All authors read and approved the final manuscript.

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Hade vi inte med en tabell 1 tidigare med all demografi + volymer och biomarkördata? Tror att det vore bra att ha en sådan.

Figure legends

Figure 1. Spearman's rank correlation coefficients (r_s) between different CSF related biophysical variables and CSF concentrations of (A) T-tau, (B) P-tau, (C) Albumin ratio, (D) Albumin, (E) sAPP α , (F) sAPP β , (G) A β 38, (H) A β 40, and (I) A β 42. Significant correlations are highlighted in grey and the error bars represent the 95% confidence interval.

Abbreviations: ICP, intracranial pressure; IC-SA, intracranial subarachnoidal.

Figure 2. Scatter plots of (A) albumin ratio versus spinal CSF volume, (B) A β 38, and (C) A β 40 versus ventricular CSF volume.